Original Research Article   https://doi.org/10.20546/ijcmas.2019.806.026

In-vivo Assay of *Escherichia coli* Microorganisms in a Live Organ using Voltammetric Microprobe

Suw Young Ly*, Chaeyun Lee,Yun Ji Kim, Min Ji won, Sih Yun Jun, Yun June Hwang,Seung Ki Kim, Seung Jun Lee, Kyung Lee

Biosensor Research Institute, Seoul National University of Science and Technology 172 Gongreung 2 dong, Nowon gu, Seoul, South Korea 139-743

*Corresponding author

A B S T R A C T

The presence of Escherichia coli (EC) microorganisms in live organs can cause foodborne illnesses and food-related deaths. Here, EC assay was performed using a microcopper three-electrode (MCE) system, where a handmade MCE was used as a working electrode and Ag/AgCl as reference and platinum counter electrodes. Under a 1.0-ml EC standard, the diagnostic optimum conditions were sought. The analytical oxidation potential was obtained at -0.2 V via positive scan. Under these conditions, the stripping linear working range was attained with 0.2-0.7 mg/mL EC variations. A statistic relative standard deviation of 6.78% (n=13) was obtained by 1.0 mg/mL EC using 0.0 sec accumulation time. Under optimum conditions, the detection limit was 0.6 mg/mL. Here, the diagnostics were explored real-time in the blood vascular system of a live frog. Moreover, which probe can be used for in-vivo clinical application in animal organs (heart, colon, lungs, and gallbladder) was determined as the patient's peak current increased a hundred times more than in the negative tissue. The sensing time was only 30 sec. This method is simpler than the common PCR amplification, electrophoresis, and photometric detection methods and can be useable for fluorescence analytical catheter probe.

Introduction

Escherichia coli (EC) and its analogous microorganisms attack the human organ systems and cause foodborne illnesses, food-related deaths (1), and hemolytic uremic syndrome (2). EC is estimated to cause approximately 73,000 illnesses and 61 associated deaths per year in the United States (3). Assays of EC and its analogous microorganisms are particularly important for live cells and in the blood vascular system (4).

EC contamination also resides in the blood and in the internal vascular organ, but it very rarely remains in the body systems. Assays for related diseases demand very sensitive diagnostic detection limits (DL) within molar ranges. The most common recently developed methods depend on polymerase chain reaction (5) and photometric luminescence detection methods, such as polymerase chain reaction (PCR) amplicons of a microsphere agglutination assay (6), surface plasmon resonance biosensor (7), microsphere

agglutination assay (8), multiplex PCR (9), multiplex real-time PCR assay (10), and enzyme-linked immunomagnetic chemiluminescence (11). Some of these PCR methods, however, require complicated DNA amplification, electrophoresis gel separation, and photometric detection, which cannot be used for in-vivo and real-time organics, and whose diagnostic detection limit is very high. For these reasons, a better and simpler voltammetric method was sought herein. The electrochemical systems were made simpler (12), and the experiment time was made shorter. Moreover, microsensor probe (13) can be used for the in-vivo vascular system (14,15) and for blood detection (16). In this study, the three-electrode system was used, with a micro-type copper electrode employed as an expensive working electrode and Ag/AgCl as reference and platinum counter electrodes. The study results can be directly applied to live organs real-time.

Materials and Methods

Systems, Reagents, Probe Fabrication, and Bacteria

The instrumental system that was used in this study was computerized handheld voltammetrics, which was carried out at the authors' institution using the bioelectronics-2 system, with a 2.4 V potential range, 2 mA current range, 10 pico A measuring current, and 5"4"1" typical cellular-phone dimensions. The MCE three-electrode probe was prepared using a 0.3-mm-diameter and 10-mm-long copper wire. The probe system was connected with a 0.5-mm-diameter copper wire to the voltammetric measurement system, whose sensors were used as Ag/AgCl reference electrode and platinum counter electrode, respectively, instead of the expensive ones. The supporting electrolyte was prepared with 0.01 M NaCl. All the other reagents were of analytical grade. Electrolyte voltammetry was

carried out at an open circuit. A common-type glassy carbon (GC) electrode was used with 3.5 mm graphite. All the electrolytes were obtained from Merck. Highly purified water was prepared through three-time distillation, using 18 $M\Omega cm^{-1}$ Milli-Q Ultra-Pure Water System (Millipore, Bedford, USA). The three-electrode system was immersed in a 1.0-mL electrolyte solution. All the experiments were performed under these conditions and at room temperature. EC was obtained from these authors' research center. The cultures were performed on tryptic soy agar slants and plates. Cultures for the ECs were grown for 20 h at 37˚C, with aeration, and were serially diluted tenfold in sterile 10 mmol/l phosphate-buffered saline, with a pH of 7.0. The number of CFUs was counted and was determined to be 3×10^{2}-4×10^{2} CFU/ml.

Results and Discussion

Cyclic and stripping effects on the EC microorganisms

Via GC probe, the cyclic reduction potentials were compared using the MCE electrode. Figure 1(A) shows the positive EC constant; active direction was performed from 2.0 V oxidation scan to -2.0 V switching potential. Under GC conditions, the horizontal voltammogram had no signal, and no peak current was obtained, and under fixed conditions, MCE probe was inserted in the same solution, and an identical cyclic scan was performed for the negative direction from the 2.0 to -2.0 V switching potentials. Shown herein are the results of the sigmoid voltammogram, which obtained -0.1 V oxidation and -0.5 V reduction with a $0.72x10^{-4}$A peak current. MCE probe obtained more sensitive voltammograms and is thus applicable to stripping voltammograms. The high CV ranges and stripping effects were thus examined using the same electrolyte. Figure 1(B) shows the

CV results for the EC variations, where the spiking range was 0.5-3.5 mg/ml add. In these voltammograms, the first curve is electrolyte blank, which is simple and does not show any peak. The next voltammogram was at 0.5 mg/ml spike, where -0.1 V oxidation potential with 0.1833×10^{-4}A current was obtained, but where no reduction current appeared. Sequential spiking was performed for 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg/ml EC, and the oxidation current was increased to 0.401-0.890×10^{-4}A. The linear working curve was y=0.243x+0.074 and the relative standard deviation was R^2=0.9654. These equations are applicable to high ranges, and more sensitive stripping was performed under optimum parameters. The anodic and cathodic strippings were examined using 0 sec accumulation time. The final voltammograms are shown in Figure 1(C), but as can be seen therein, the cathodic stripping was not sensitive and exact, and a sharp peak was obtained only in the anodic stripping. The working curve was obtained at 7-point spiking, where the linear equation was obtained, which can be used for diagnostic applications. Here, the peak potential can be applied to diagnostic EC infection, but more sensitive detection methods were studied using SW stripping. More sensitive working ranges were subsequently arrived at.

Analytical SW optimizations of MCE

Under the 1.0-mL electrolyte solution with a 0.1 mg EC spike, a -2.0 V initial potential and a 2.0 V switching potential were obtained. The SW anodic-stripping optimum parameters were examined. First, the SW accumulation times within the 0-30 sec range were used, employing 8 points. It increased continuously, and the 30 sec accumulation time showed peak height results. Thus, 30 sec accumulation time was used for all the other experiments. Under this condition, the SW parameters of frequency, increment potential,

initial potential, and switching potential were examined (data not shown). Finally, the optimum analytical SW conditions were set at 0.025 mV amplitude, 4 mV increment potential, -2.0 V accumulation potential, and 30 sec accumulation time, where MCE was very sensitive and sharp. Here, MCE was found to be suitable for the detection of EC. Using these parameters, the diagnostic working ranges, application, and statistics were examined.

Diagnostic linear range and probe stability

For organic conditions, *in-vivo* or *in-vitro* diagnostics are required for very low analytical detection limits. Thus, the ug/ml-range working conditions were sought.

Using SW oxidation scan, the diagnostic linear working ranges were examined via positive stripping. The voltammograms are shown in Figure 2(A). The first curve, representing electrolyte blank, is simple, and no signal is observed in the 1.0-mL solution. The next curve represents the 0.1 mg/ml EC spike, which was obtained at -0.2 V with a 1.66×10^{-5}A peak current, and which continued spiking from 0.2 to 0.7 mg/mL. The linear ranges appeared in the oxidation scan, where the peak current was varied from 1.93 to 2.43×10^{-5} A, and where no reduction current was obtained. The slope sensitivity was $\triangle x / \triangle y$=0.0012, and the analytical precision was R^2=0.812, which indicate that the method can be used for in-vivo or in-vitro applications. Under these conditions, the statistic detection limits were carried out using KSb/m (k=3, n=15, m=$\triangle x / \triangle y$). The detection limit was attained at 0.6 mg/mL (S/N=3) SW, which shows that the stripping is more sensitive than that shown in the CV results. Under these conditions, the new probe stability was examined with the replicated 15th stripping at the 1.0 mg/ml EC spike. Figure 2(B) shows the peak high, where the

first peak is the electrolyte and where the linear range oscillated, and from which 0.830% RSD was obtained. These probes are highly reproducible and usable for EC diagnosis, making them suitable for *in-vivo* diseases.

So that the results of this study could be used for in-vivo or in-vitro diagnostics, live-frog application was performed using a 150-mm-long healthy body weighing 120 g.

The developed method was used to measure healthy and contaminated human blood using SW stripping voltammograms, which were examined using the optimum parameters shown in Figure 3(A), although the healthy

blood was very simple and linear. No current was observed when the aforementioned parameters were used at 30.0 sec accumulation time. Under these conditions, the patient's blood spiking was examined under a newly prepared cell system. The patient's spiking voltammogram was obtained $(0.7 \times 10^{-5} A)$, which was much larger than the blank noise $(0.2 \times 10^{-5} A)$. This indicates that it can be used for diagnosis.

Here, a more advanced application was made, using live organs. Illness detection was performed using a 120-g simulated frog that was narcotized via 5-mg/ml EC injection in the hind-leg muscle.

Fig.1 (A) Cyclic voltammetric probe effects on the 1.0 mg/ml EC constant. The horizontal CV curve represents the GC electrode, and sigmoid is the MCE probe, with a 2.0 V initial potential and a -2.0 V switching potential. (B) CV concentration effects for the 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg/ml EC variations. (C) Stripping concentration effects for the 0.5-3.5 mg/ml EC variations, using optimum parameters
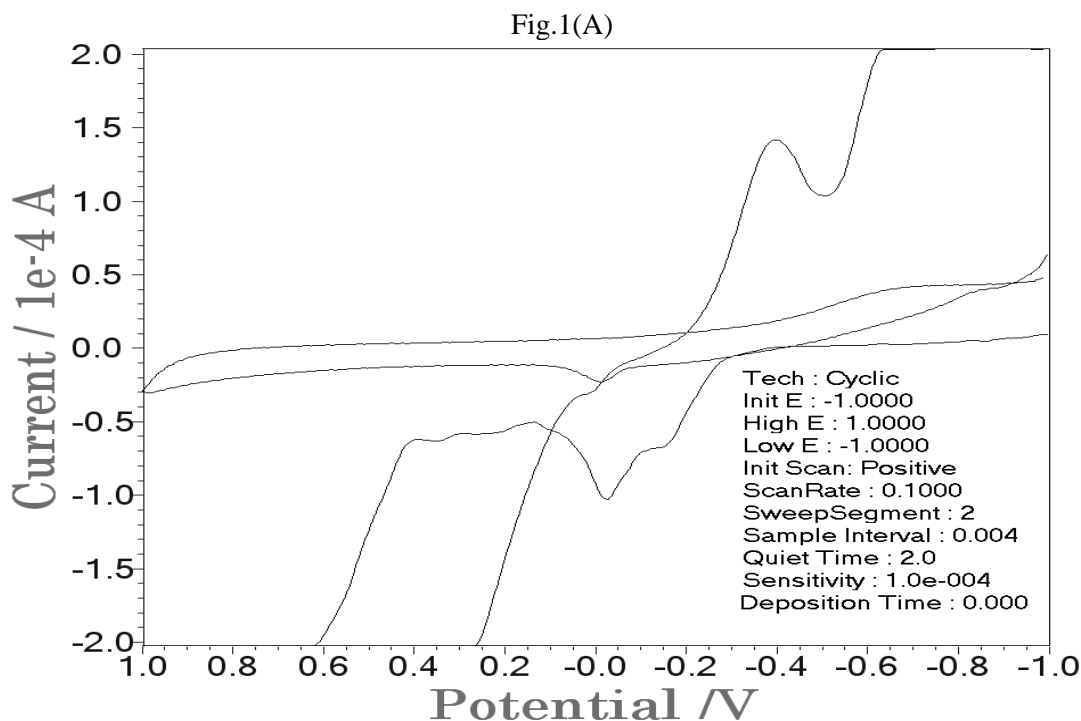

Fig.1(A)

Tech : Cyclic
Init E : -1.0000
High E : 1.0000
Low E : -1.0000
Init Scan: Positive
ScanRate : 0.1000
SweepSegment : 2
Sample Interval : 0.004
Quiet Time : 2.0
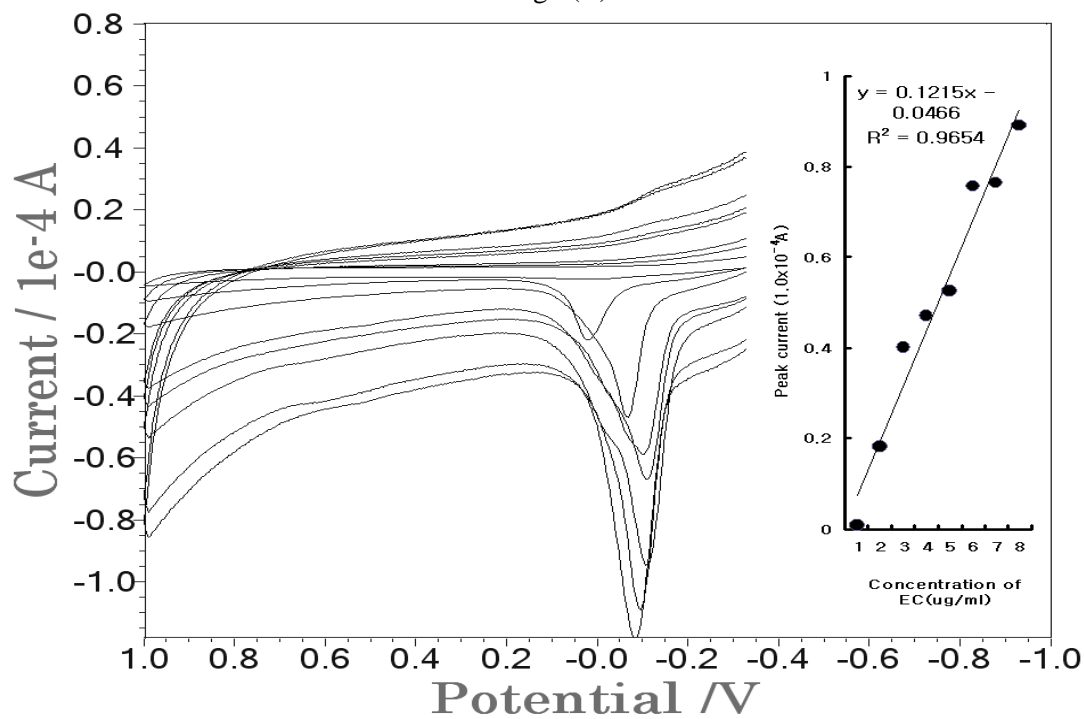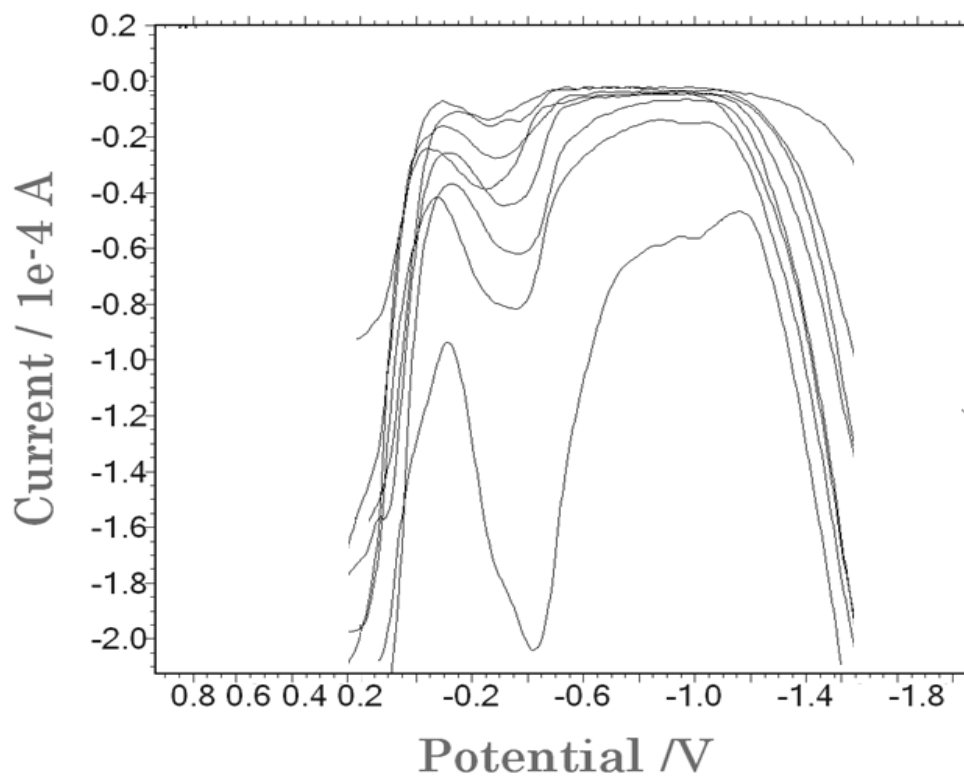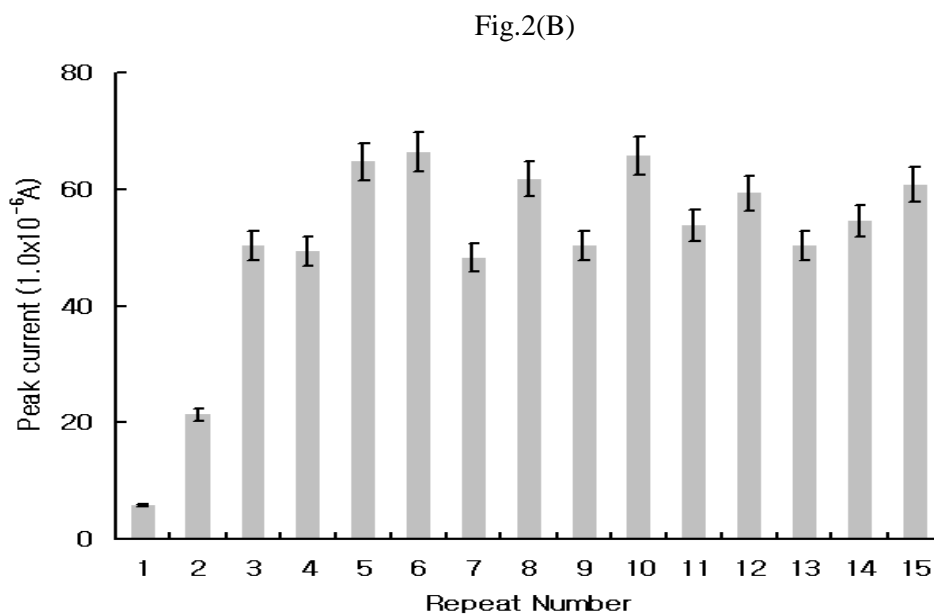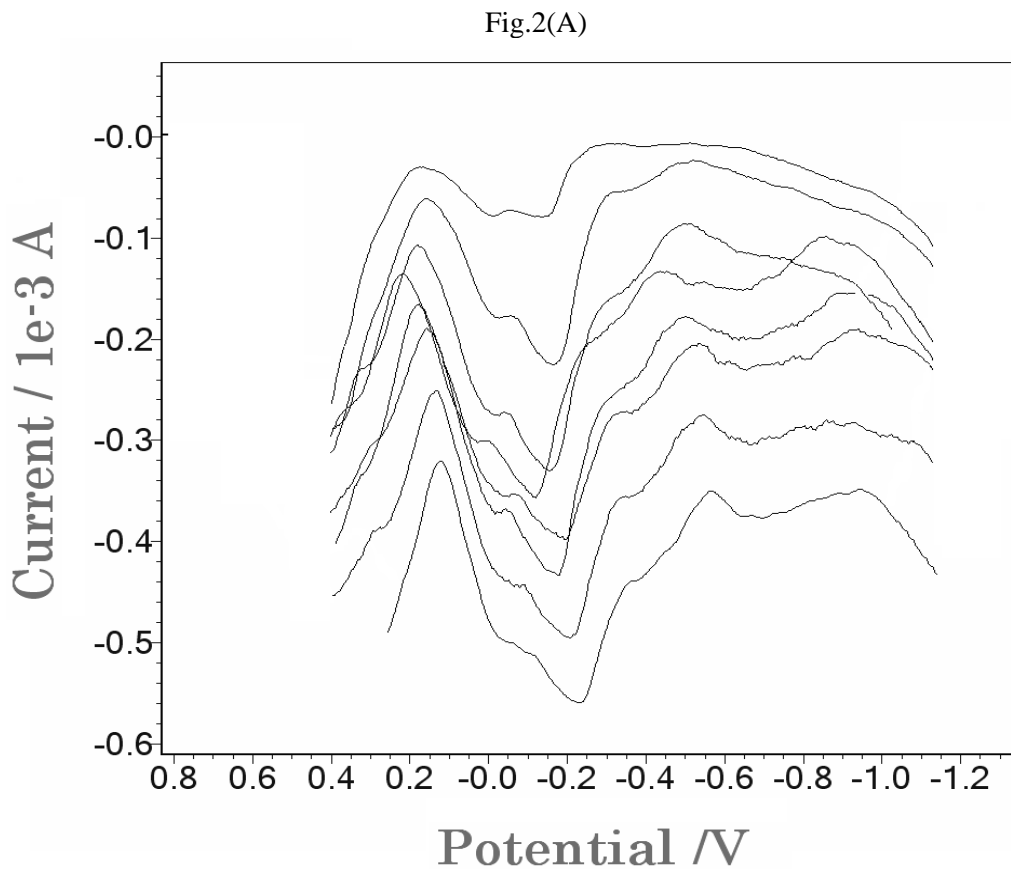Sensitivity : 1.0e-004
Deposition Time : 0.000

Fig.1(B)

Fig.1(C)

Fig.2 (A) SW linear working ranges of the 0.1 , 0.2, 0.3, 0.4 0.5, 0.6, and 0.7 mg/mL EC spikes in a 1.0-ml electrolyte with a 0.025 mV SW amplitude, 15 mV SW frequency, 4 mV increment potential, -2.0 V accumulation potential, and 30 sec accumulation time. (B) SW statistic MCE stability at the 1.0 mg/ml EC constant using 30 sec accumulation time and the parameters in (A)

Fig.2(A)

Fig.2(B)

Fig.3 (A). Diagnostics of healthy and contaminated blood using 1.0 ml human serum. Under in-vivo conditions, diagnostic applications were made for the frog's stomach (B) and kidney (C), using the optimum parameters of the CV scan
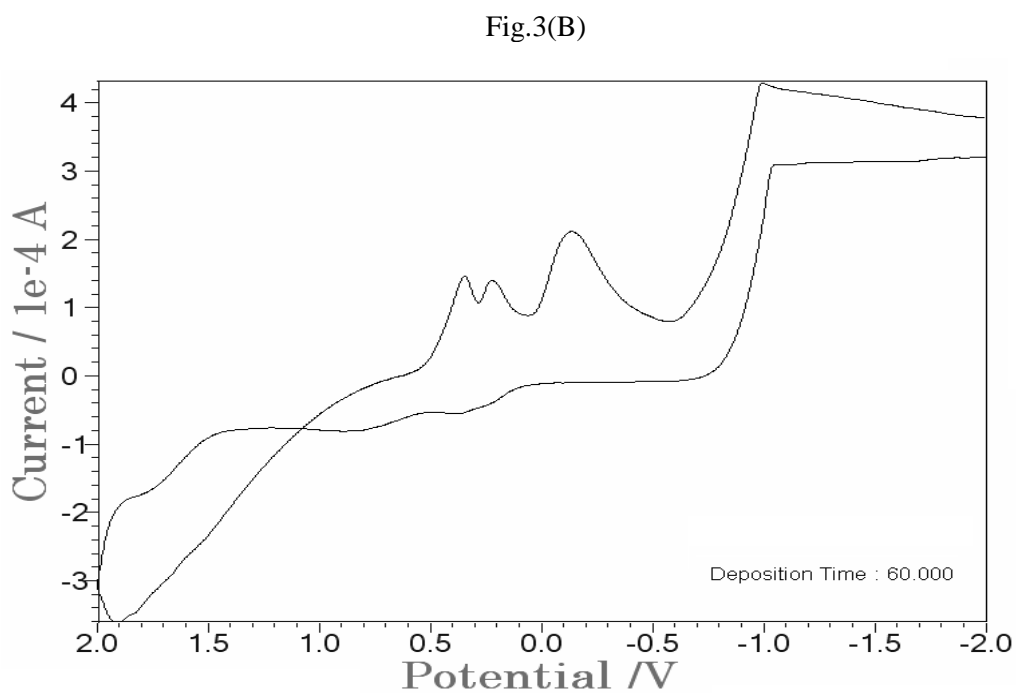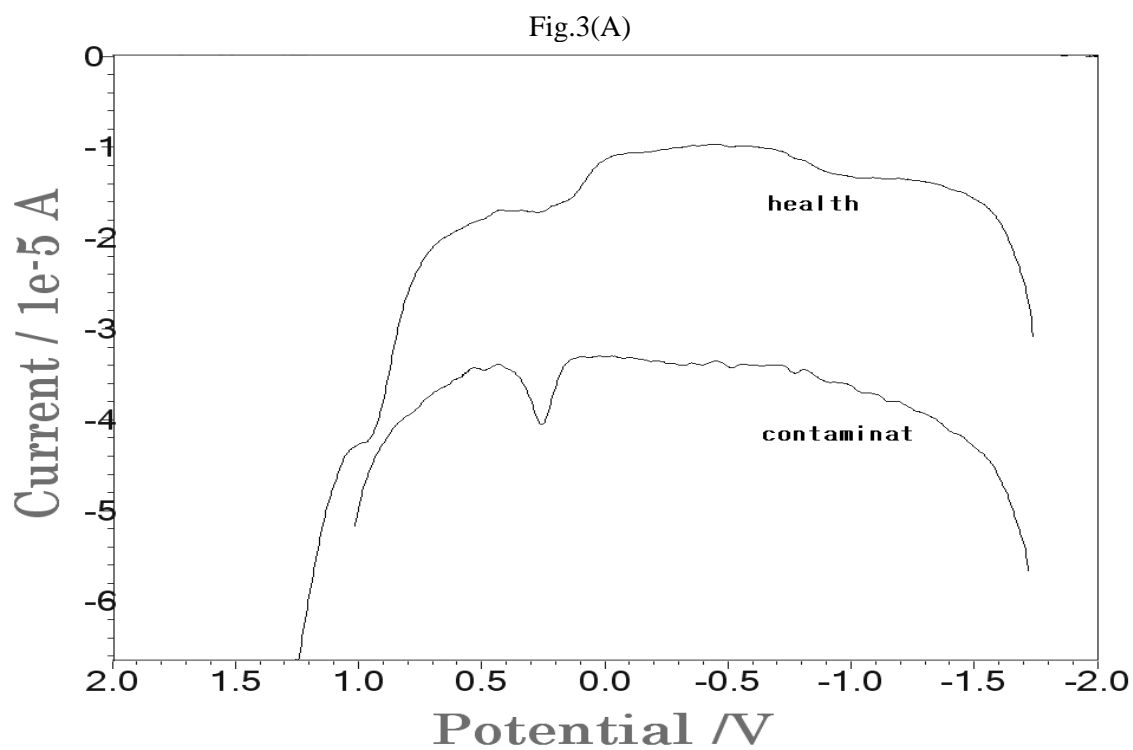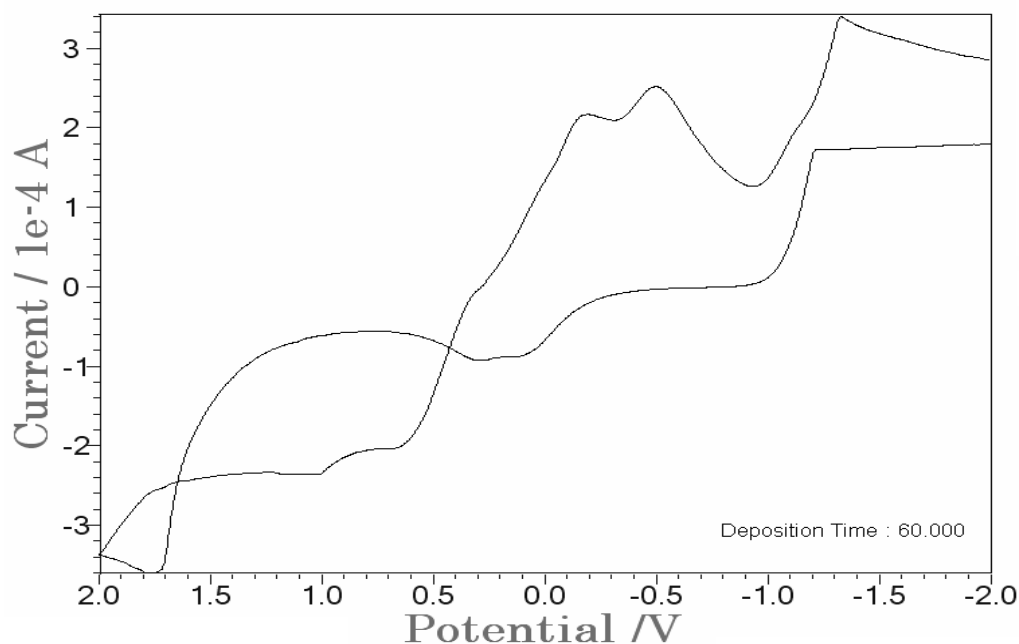
Fig.3(A)

Fig.3(B)

Fig.3(C)

Thirty minutes later, dissection was performed, where, under live conditions, the counter and reference electrodes were inserted into the leg muscles, then the working probe was connected to the lungs, bladder, kidney, large intestine, and stomach. Detection was carried out using only 30 sec accumulation time. Figure 3(B) and (C) show the 0.0 V reduction peaks of 7.2×10^{-4} and 1.4×10^{-4} A, respectively. The diagnostic biosensor was successfully applied for the detection of the amounts of EC trace labels in human serum and in live frog organs (stomach and kidney). The developed method can also be used in other fields that require diagnostics in humans.

In conclusion, after the comparison of the common-type GC and the modified macro-type MCE via CV and SW, it was found that the MCE with SW was more effective in detecting trace microorganisms in EC assay. Under optimized conditions, the diagnostic detection limit was attained at 0.6 mg/ml despite the use of inexpensive electrodes and a short experiment time of only 30.0 sec.

Moreover, the same reference and auxiliary electrodes were used (working copper electrode), and the electrolyte solutions were very small. The working range was 0.1-0.7 mg/ml. The developed probe was applied to direct liver assay in organs, and the results of the application show that it can be used under in-vivo non-treated conditions. It can also be used in other fields that require diagnostic assay in human body systems.

References

1. Nadezhda V. Kulagina, Kara M. Shaffer, George P. Anderson, Frances S. Ligler, Chris R. Taitt.Antimicrobial peptide-based array for Escherichia coli and Salmonella screening, Analytica Chimica Acta 575 (2006) 9–15.

2. A. Abdulmawjood, M. Bulte, N. Cook, S. Roth, H. Schonenbrucher, J. Hoorfar, Toward an international standard for PCR-based detection of Escherichia coli O157 Part 1. Assay development and multi-center validation, Journal

of Microbiological Methods 55 (2003) 775-786.

3. Chien Sheng Chen, Richard A. Durst, Simultaneous detection of Escherichia coli O157:H7, Salmonella spp. and Listeria monocytogenes with an array-based immunosorbent assay using universal protein G-liposomal nanovesicles, Talanta 69 (2006) 232-238.

4. T. Ziegler, N. Jacobsohn, R. Fünfstück, Correlation between blood group phenotype and virulence properties of Escherichia coli in patients with chronic urinary tract infection, International Journal of Antimicrobial Agents 24S (2004) S70-S75

5. Nathalie Y. Fortin, Ashok Mulchandani, Wilfred Chen, Use of Real-Time Polymerase Chain Reaction and Molecular Beacons for the Detection of Escherichia coli O157:H7, Analytical Biochemistry 289, 281-288 (2001)

6. . Shaw Jye Wu, Alex Chan, Clarence I. Kado, Detection of PCR amplicons from bacterial pathogens using microsphere agglutination, Journal of Microbiological Methods 56 (2004) 395–400.

7. John Waswa, Joseph Irudayaraj, Chitrita DebRoy, Direct detection of E. Coli O157:H7 in selected food systems by a surface plasmon resonance biosensor, LWT 40 (2007) 187–192.

8. ShawJye Wu, Alex Chan, Clarence I. Kado, Detection of PCR amplicons from bacterial pathogens using microsphere agglutination, Journal of Microbiological Methods 56 (2004) 395-400.

9. Son Radu, Ooi Wai Ling, Gulam Rusul, Mohamed Ismail Abdul Karim, Mitsuaki Nishibuchi, Detection of Escherichia coli O157:H7 by multiplex PCR and their characterization by plasmid profiling, antimicrobial resistance, RAPD and PFGE analyses, Journal of Microbiological Methods 46, 2001. 131–139.

10. Vijay K. Sharma, Evelyn A. Dean-Nystrom, Detection of enterohemorrhagic Escherichia coli O157:H7 by using a multiplex real-time PCR assay for genes encoding intimin and Shiga toxins, Veterinary Microbiology 93 (2003) 247–260.

11. Andrew G. Gehring, David M. Albin, Peter L. Irwin, Sue A. Reed, Shu I Tu, Comparison of enzyme-linked immunomagnetic chemiluminescence with U.S. Food and Drug Administration's Bacteriological Analytical Manual method for the detection of Escherichia coli O157:H7, Journal of Microbiological Methods 67 (2006) 527–533.

12. Suw Young Ly, Jung Eun Kim, Woo Yeon Moon, Diagnostic assay of carcinoembryonic antigen tumor markers using a fluorine immobilized biosensor with handmade voltammetric circuit, European Journal of Cancer Prevention 2011, Vol 20, 58-62.

13. Suw Young Ly, Hwa Jin Heo, Min Jung Kim, Real Time Analysis of Neurotransmitters in the Brain Using a Micro Electrode System, Current Neurovascular Research, 2010, 7, 32-38

14. Suw Young Ly, Diagnosis of copper ions in vascular tracts using a fluorine doped carbon nanotube sensor, Talanta 74 (2008) 1635–1641.

15. Suw Young Ly, Jong Keun Kim, Simultaneous Real-Time Assay of Copper and Cadmium Ions by Infrared Photo Diode Electrode Implanted in the Muscle of Live Fish,

J Biochem Molecular Toxicology, Volume 23, 256-262

16. Suw Young Ly, Nam Sun Cho, Diagnosis of human hepatitis B virus in nontreated blood by the bovine IgG DNA linked carbon nanotube biosensor, Journal of Clinical Virology 44 (2009) 43–47.

How to cite this article: